J PREV MED HYG 2015; 56: E190-E195

ORIGINAL ARTICLE

Occurrence of Acrylamide in breakfast cereals and biscuits available in Italy

R. CAPEI, L. PETTINI, A. LO NOSTRO, G. PESAVENTO
Department of Health Sciences, University of Florence, Italy

Key words

Acrylamide • Biscuits • Breakfast cereals

Summary

Introduction. *Acrylamide, produced during thermal processing of carbohydrate-rich foods, is classified as "probably carcinogenic to humans" by the International Agency for Research on Cancer.*
Objective. *The aim of this study was to determine acrylamide levels in biscuits and breakfast cereals considering the widespread use of these products for all ages.*
Method. *Acrylamide determination was carried out in 56 samples by HPLC-UV technique.*
Results and discussion. *The results showed a considerable variability in the contents of acrylamide in the samples analysed, most likely due to differences in industrial processing and ingredients. The percentages of contaminated samples tested were very high (95.5% of the biscuits and 75% of the breakfast cereals) with a wide range of contamination: from 30 µg/kg to 940 µg/kg. Our results showed that 22.7% of biscuits and 33% of breakfast cereals exceeded the indicative values recommended by EC 2013/647 set at 500 µg/kg and 200-400 µg/kg (according to the composition) respectively.*
Conclusions. *Our findings suggest concern about the risk for human health.*

Introduction

Acrylamide (AA) was first detected in heat-treated foods, especially potato chips, at high concentrations (mg/kg) by a group of Swedish researches [1-3]. This finding aroused great interest in all the world because of the well-known toxicity of AA, classified as "probably carcinogenic to humans" (Group 2A) by the International Agency for Research on Cancer [4] and as an A3 carcinogen (confirmed animal carcinogen with unknown relevance to humans) by *The American Conference of Governmental Industrial Hygienists* (as cited in *Agency for Toxic Substances and Disease Registry* [5]). Experimental studies in animals have shown that acrylamide causes chronic nephrotoxicity, adverse reproductive effects, and is characterized by genotoxic and carcinogenic activity mainly in glands: thyroid, mammary, adrenal, pituitary etc. [6-9]. The genotoxic activity of acrylamide seems to be partly mediated by its main metabolite, glycidamide [10-12], which is mutagenic [13-15].
The risk of cancer due to AA intake is unclear; the epidemiological studies on humans up to present have reported conflicting data and do not provide any substantial evidence that dietary exposure to AA is associated with cancer in humans, therefore more data are necessary to better assess the risk from dietary intake [7, 16], whereas the neurotoxicity activity of AA in humans is rather well known in accidental or occupational exposure through inhalation or dermal absorption [17].
AA is produced during thermal processing, at temperatures of 120°C or higher and low moisture, mainly via the Maillard reaction, responsible for the flavour and colour of cooked foods, between amino acids (mainly free asparagine) and reducing sugars, such as glucose and fructose [1, 7, 18, 19]. However, several other pathways and precursors have been proposed to contribute to AA formation as reviewed by Keramat et al. [20]. Most of AA is formed rapidly during final phases of baking, grilling or frying processes [21] of carbohydrate-rich foods such as fried potato and bakery products, biscuits, breakfast cereals, cocoa and coffee.
After ingestion, AA is rapidly absorbed and distributed to different body tissues and organs such as muscle, liver, heart, thymus, brain, kidneys, placenta and breast milk, thus also representing a significant risk to the health of foetus and infants [22, 23].
AA is metabolized by the liver to the reactive genotoxic epoxide, glycidamide. AA and glycidamide are then conjugated with glutathione, following the classical detoxification scheme, and eliminated in urine as mercapturic acid derivatives [24]. Since the urinary excretion is about 50% in the first 24 hours and 90% within 7 days, the AA metabolites could be used as an intake biomarker as recent exposure to AA [16, 23, 25, 26].
As stated above, with concern for the potential risk on human public health, and in particular in babies and adolescents, since 2003, at the request of the European Commission [27], many studies have been carried out worldwide both on the development of analytical methods and on the determination of AA in food. According to this Recommendation, furthermore, Member States should carry out AA monitoring annually and provide

the relative data to the *European Food Safety Agency* (EFSA) by June 1st of the following year.
The recent EFSA Scientific Report [28] showed the results of the monitoring of AA in specific food categories from 2007 to 2010 and the estimation of the trend in AA levels by comparing results from 2007, 2008, 2009 and 2010 in main food categories and sub-categories. Data collected showed only a few changes in the trend of AA values: a decrease in 'processed cereal based foods for infants and young children' and an increase in 'coffee and coffee substitutes'. The highest AA levels have been found in fried potato products, bread, bakery products and coffee. In 2010 the AA mean values ranged from 31 μg/kg in 'other processed cereal based foods for infants and young children' to 1350 μg/kg in 'coffee substitutes', while breakfast cereals and biscuits had mean values of 138 μg/kg and 289 μg/kg respectively. The last Scientific Opinion on AA in Food by EFSA [29] published in 2015, confirms that the major contributing foods to AA exposure are potato products, coffee and bakery products (biscuits and bread) and relates that dietary AA intake is estimated in the range of 0.3-1.9 μg/kg b.w. per day for the general population, and for the 95th percentile exposure from 0.6 to 3.4 μg/kg b.w. per day. EFSA concludes that "AA in food potentially increases the risk of developing cancer for consumers in all age groups".
Within the *European Prospective Investigation into Cancer and Nutrition* (EPIC) study, Freisling et al. [30] estimated the AA intake in 10 European countries: the study, involved a total of 39 994 participants, aged 35-74, showed that the mean intake ranged from 12 to 39 μg per day for women and from 13 to 47 μg for men.
Moreover, a substantial probability that the intake by children and adolescents is remarkably higher than that of adults must be considered, due to their more frequent consumption of certain foods such as fried potato products and biscuits.
A large group of products contributing to AA intake is cereal-based food. In Italy, to our knowledge, the data regarding dietary AA exposure principally concerned potato chips, which are estimated to contribute about 30% of the total diet [31-33] and coffee [34], whereas cereal-based foods have been investigated less in recent years.
A maximum limit for AA in drinking water (0.5 μg/L) has been established by WHO [35], but no limit is established in food.
In 2011, the European Commission [36] established indicative values, as amended by Recommendation EC of 2013 [37], for the food categories cited in Recommendation EC of 2010 [38] including 'french fries', 'potato crisps', 'soft bread', 'breakfast cereals', 'biscuits, crackers, wafers, crisp bread and similar', 'roast coffee', 'instant coffee', 'biscuits and rusks for infants and young children', 'processed cereal based foods for infants and young children' and 'baby foods, other than processed cereal based foods', with the exception of the products for home cooking and 'other products'. This Recommendation stresses that 'these values are to be understood as indicative of the need for an investigation and not as safety thresholds'.
In Italy, the second largest item of expenditure in the cereals derivatives segment is products for breakfast (30% of total). 95% of Italians buys biscuits at least once a year. In 2013 an increase in the volume of purchases of bakery products was observed (+ 3.2%) compared to 2012 [39]. Furthermore, although in Italy the breakfast cereals market is not yet as developed as in other countries, over the last few years we have witnessed a process of strong sales growth in volume. In particular, from 2004 to 2007, the share of household spending on breakfast cereals has increased. In 2007, fortified cereals and grains held the vast majority of sales quotas by volume, with 46% and about 44% respectively [40].
The occurrence of AA in commercially available food is a matter of interest for public health due to continuous and prolonged exposure to a probable carcinogenic substance. As the ingestion of contaminated food is the primary route of potential human exposure to AA, the aim of this research was to determine AA levels in cereal-based food, in particular biscuits and breakfast cereals, considering the widespread use of these products for all ages.

Materials and methods

Samples

A total of 56 samples of biscuits and breakfast cereals of the most consumed brands in Italy (17 and 5 respectively) were randomly collected in the two major supermarkets in Florence, as indicated by the European Commission Recommendation n° 307 [38], and tested for AA. The samples collected included 12 breakfast cereals and 44 biscuits whose composition consisted of maize, rice, malt, oatmeal, wheat, spelt, barley, rye and cocoa in different combinations.

AA determination

AA determination was carried out following the method of Wang et al. [41] except for some modifications both in the extraction procedure and in the instrumental working conditions [42]. The HPLC method adopted is fast and cost-effective and it can be applied to screen the occurrence of AA in food samples ensuring a good degree of reliability.

Instruments and reagents

All organic solvents, methanol and acetonitrile, were from J.T. Baker (Deventer, Holland) HPLC-grade. Water was purified by a Milli-Q-RO4 water purification system (Millipore, Bedford, MA, USA) with a resistivity of 10 MΩ·cm. The standard AA was purchased from Fluka (Deisenhofen, Germany). Potassium hexacyanoferrate (II) trihydrate and zinc sulfate heptahydrate were obtained from Merck (Darmstad, Germany). Oasis HLB 200 mg/6 mL SPE cartridges were obtained from Waters (Milford, MA, USA). Bond Elut-Accucat (200 mg/3 mL) SPE cartridges were purchased from Varian (Chi-

cago, IL, USA) and used with a SPE vacuum manifold (Visiprep, Supelco, Bellefonte, PA, USA). Amber glass auto-sampler vials with septum screw caps were obtained from Agilent Technologies (Wilmington, DE, USA). The analytical column (Atlantis dC18, 250 × 4.6 mm/5) with pre-column and syringe filters (0.45 µm PVDF) were from Waters (Milford, MA, USA).
A standard AA stock solution (1.0 mg/mL) was prepared by dissolving 1.0 mg of AA in 1.0 mL of Milli-Q water. To weigh AA, an electronic analytical balance (Radwag, PCE group, 0.01 mg Radom, Poland) was used. The AA stock solution was diluted in amber glass volumetric flasks to prepare calibration standards at 50, 100, 200, 500, 800 and 1000 ng/mL respectively and stored at 4°C until use.

Sample preparation

100 g of each sample was finely pulverized, homogenized and dehydrated at 103°C for 4 hours, prior to sampling. Two 1.0 g solid samples were accurately weighed into centrifuge tubes.
A volume of 10 µl of the internal standard solution, AA at 100 µg/mL, was added to one centrifuge tube for recovery study. AA was extracted by adding 8 mL of water. The samples were shaken for 3 min on a Vortex and centrifuged (15 min at 3100 x g). The aqueous phase was collected and the extraction process was repeated twice, each time adding 2 mL of water, shaking for 2 min, and centrifuging for 15 min at 3100 x g. Aliquots of 1 ml of a 0.355 mol/l potassium hexacyanoferrate (II) trihydrate solution (Carrez I) and 1 ml of a 1.04 mol/l zinc sulfate heptahydrate solution (Carrez II) were added to the supernatant for protein precipitation. The samples were shaken for 2 min, kept cool at 4°C for 30 min, then centrifuged (15 min at 3100 x g). The samples were filtered through a 0.45 µm Millex syringe filter. Oasis HLB SPE cartridges and Bond Elut-Accucat SPE cartridges, conditioned with methanol and washed by water, were used in sequence to remove the first eluted fraction containing interfering substances. The final eluted fractions were concentrated to 1 mL by evaporation under vacuum at 45°C kept in amber glass vials at 4°C before injection.

HPLC-UV analysis of AA

HPLC-UV analysis was performed with a HPLC instrument equipped with a vacuum degasser, a binary pump, an autosampler, and a DAD detector (Waters 1525 binary pump /Waters 996 PDA detector), at room temperature. The injection volume of calibration standards and sample extracts was 20 µl; AA was detected at two UV wavelengths of 200.5 and 210 nm. An isocratic elution was performed at a flow rate of 0.5 ml/min; the mobile phase consisted of water/acetonitrile 96:4 (v/v).
The retention time of AA was approximately 10.1 min, and the total run time was 35 min.
Under these chromatographic conditions, AA and the food components in the samples were all separated and eluted. AA content is resulting from an average of three measurements and the relative standard deviation of each sample was below 10%.
The AA recovery was on average 87%.
Table I reports the analytical parameters calculated in the range of 0.05-2 µg/g. The Limit of Detection (LOD) (calculated using the criterion signal to noise, s/n, of 3:1) and Limit of Quantification (LOQ) (calculated using the criterion s/n of 7:1). The intra-day and inter-day measurements show a good repeatability and reproducibility of the analytical methodology.

Results and discussion

The results of the survey are shown in Tables II and III. According to Commission Recommendation n° 307 (2010) the main ingredients are reported for each sample. Our results are comparable to those of other studies [43-45]. Of 56 samples tested, only five had AA concentrations below the detection limit (10 µg/kg). The percentage of positive samples (≥ LOD) we detected in both kinds of food analysed was higher in biscuits (95.5%) than in breakfast cereals (75%) with a wide range of contamination and a considerable variability, as indicated from the high levels of standard deviation (294.1 and 287.75 respectively), also within each brand especially in biscuits (Tab. II). In detail, the standard deviation of the samples of brand 6 was 297.85 (AA levels ranging from < LOD to 820 µg/kg) while that of brand 17 was 298.57 (AA levels ranging from 30 µg/kg to 700 µg/kg). The biscuits of three brands (number 1, 9 and 16) showed the lower values, ranging from 30 to 50 µg/kg, from < LOD to 30 µg/kg and from 30 to 40 µg/kg respectively.
In the biscuit category, compared to that of breakfast cereals, we noted also the highest value, in spite of the fact that the average value and the median are slightly lower. The range of contamination of biscuits was from 30 µg/kg to 940 µg/kg. Only ten samples (22.7%) of biscuits showed a concentration above 500 µg/kg, set as the indicative value recommended [37]. The maximum level of 940 µg/kg was found in the sample of brand 14 (Tab. II), probably due to the industrial process conditions and to the presence of many different ingredients, such as

Tab. I. Analytical data of the acrylamide standard solution analysed by HPLC-UV: linear regression equation (peak area/conc.) and correlation coefficient (R^2) calculated in the linearity range 0.05-2 µg/g at wavelengths 210 and 200.5 nm; limit of detection (LOD, s/n = 3/1) expressed as µg/kg; limit of quantification (LOQ, s/n = 7/1) expressed as µg/kg; intra-day RSD and inter-day RSD (n = 3).

Linear regression equation	R^2	LOD (µg/kg)	LOQ (µg/kg)	Intra -day [a] RSD	Inter-day [b] RSD	Wavelength
y = 116616 x -4415. 8	0.998	10	28	3-6.6	6.8	210 nm
y = 250771 x -2640. 9	0.999	10	28	4.2-7.2	1.9	200.5 nm

[a] Values are referred to six independent samples analyzed in one day
[b] Values are referred to six independent samples analyzed three times in three different days

Tab. II. Acrylamide (AA) content (µg/kg) in biscuits.

Biscuits (N. samples)	Main ingredients [a]	AA µg/kg
Brand 1 (2)	Grain	50
	Wheat /barley	30
Brand 2 (4)	Wheat/rice/barley	66
	Wheat/oat	200
	Wheat/cocoa	200
	Wheat/maize	30
Brand 3 (1)	Wheat /cocoa	310
Brand 4 (3)	Wheat/maize/rice/oat	560
	Grain/maize/oat	840
	Grain	50
Brand 5 (1)	Wheat /cocoa	400
Brand 6 (7)	Wheat	< LOD
	Wheat/oat	300
	Whole grain	90
	Wheat	80
	Hazelnuts/ cocoa	590
	Wheat /cocoa	200
	Wheat /cocoa	820
Brand 7 (1)	Wheat	260
Brand 8 (1)	Whole grain	450
Brand 9 (2)	Wheat/maize	< LOD
	Wheat	30
Brand 10 (2)	Whole grain/oat	430
	Wheat	280
Brand 11 (2)	Grain	840
	Whole grain	720
Brand 12 (2)	Grain /cocoa	840
	Wheat	50
Brand 13 (3)	Maize/barley	280
	Wheat	190
	Wheat /cocoa	90
Brand 14 (1)	Wheat/oat/barley/ Rice/maize/rye	940
Brand 15 (2)	Wheat	100
	Wheat	180
Brand 16 (3)	Wheat /cocoa	140
	Wheat/rice	30
	Wheat	40
Brand 17 (7)	Wheat	40
	Grain	60
	Wheat /rice	30
	Grain/barley/rice/ Oats/rye	530
	Wheat /cocoa	240
	Wheat/cocoa	490
	Wheat /cocoa	700
mean [b]	297.18	
median [b]	200	
LB - UB	< LOD - 940	

[a] The first is the ingredient present in the largest quantity
[b] Results lower than LOD were assigned ½ LOD
LB: lower bound
UB: upper bound

Tab. III. Acrylamide (AA) content (µg/kg) in breakfast cereals.

Breakfast cereals (N. samples)	Main ingredients [a]	AA µg/kg
Brand 1 (1)	Maize/rice	360
Brand 2 (3)	Oat/wheat/maize/rice	< LOD
	Rice/wheat	< LOD
	Wheat/maize/rice/cocoa	220
Brand 3 (5)	Wheat/rice/oat	280
	Wheat/cocoa	60
	Maize/barley	< LOD
	Maize/oat/rice/cocoa	110
	Grain/oat/rice/cocoa	450
Brand 4 (2)	Wheat/rice/cocoa	810
	Spelt	780
Brand 5 (1)	Wheat/cocoa	80
mean [b]	263.75	
median [b]	165	
LB-UB	< LOD - 810	

[a] The first is the ingredient present in the largest quantity
[b] Results lower than LOD were assigned ½ LOD
LB: lower bound
UB: upper bound

wheat, oat, barley, rice, maize, rye. Ingredients indeed play an important role in AA formation, as different cereals have different amounts of free asparagine and reducing sugars available for the Maillard reaction [23].
EC Recommendation n° 647 [37] amended the previous indicative values for breakfast cereals according to the composition: for bran products and whole grain cereals the indicative value is 400 µg/kg, for wheat and rye based products it is 300 µg/kg, while for maize, oat, spelt, barley and rice based products the value is 200 µg/kg.
In the category of breakfast cereals tested, the AA occurrence, ranging from 60 to 810 µg/kg, is less frequent (75%) than in biscuits, although the percentage of samples with AA concentration above the indicative values recommended (33%) was higher than that stated in the EFSA report [28]. The mean value obtained was 263.75 µg/kg and the median value was 165 µg/kg; both these values were lower than those of the biscuits (297.18 and 200 µg/kg respectively) and in agreement with data recorded in Europe and other countries [7, 21, 28].
The highest values were found in two samples of brand 4, one consisting of spelt (780 µg/kg) and the other consisting of wheat, rice and cocoa (810 µg/kg): this also supports the hypothesis that the industrial process of preparation is the determinant key factor.
Of 56 samples tested, 18 (32.1%) contained cocoa: 12 (27.3%) biscuits and six (50%) breakfast cereals. None of these had AA concentrations below the detection limit, and they showed a wide range of contamination, from 60 µg/kg in a breakfast cereal to 840 µg/kg in a biscuit sample. The average value of all samples containing cocoa was 375 µg/kg and the median 275 µg/kg. In comparison to the rates related to the AA contamination in the samples without cocoa (mean = 242.6 µg/kg, median = 95 µg/kg), we suppose that the presence of cocoa

probably contributes to the increase of AA concentration. Some studies [46, 47] reported that AA concentration in cocoa is variable and can reach concentrations of about 600 µg/kg.

In the scientific report of EFSA [28] on AA levels in food from monitoring years 2007 to 2010, the average concentration of AA in breakfast cereals and biscuits ranged from 138 and 289 µg/kg to a maximum of 1290 and 5849 µg/kg respectively, and the median values are 99 and 91 µg/kg (data referring to 2010). We noted that our results show mean and median values higher than those reported by EFSA [28], while the maximum values are clearly lower.

Our results, compared to the AA indicative values recommended [37], showed that 22.7% of biscuits and 33% of breakfast cereals exceeded the indicative values set at 500 µg/kg for biscuits and from 200 to 400 µg/kg for breakfast cereals, depending on the composition. Our findings are interesting to evaluate the impact of AA on dietary intake as the overall consumption of breakfast cereals by Italian consumers is 14.8 g/d, while the overall consumption of biscuits is more relevant because it is rated at 27.3 g/d. For food consumption data Authors referred to a survey carried out by the Italian Institute for Nutrition (INRAN) [48] that is highly representative of Italian population for age, gender, geographical area and the wide number of subjects involved.

Conclusions

In conclusion our data, not unlike that from the most recent research carried out in Italy and other countries, showed widespread contamination by AA with high frequency of positive samples (91% of all samples tested ≥ LOD) and considerable variability in concentration values, most likely due to industrial processing and the presence of various ingredients.

The good news is that 41.1% of samples tested, 40.9% biscuits and 41.7% breakfast cereals, showed an AA concentration below 100 µg/kg, which can be considered a low value, even if low concentrations may not imply zero risk, especially in view of neoplastic effects based on animal evidence.

Since cereal based products are contributing substantially to human exposure, reducing AA levels in foods should be a high priority to decrease the risk for human health.

References

[1] Friedman M. *Chemistry, biochemistry, and safety of acrylamide: A review.* J Agr Food Chem 2003;51:4504-26.

[2] Stadler RH, Blank I, Varga N, et al. *Acrylamide from Maillard reaction products.* Nature 2002;419:449-50.

[3] Tareke E, Rydberg P, Karlsson P, et al. *Analysis of acrylamide, a carcinogen formed in heated foodstuffs.* J Agr Food Chem 2002;50:4998-5006.

[4] International Agency for Research on Cancer [IARC]. *Acrylamide.* In: *IARC monographs on the evaluation of carcinogen risk to humans.* Some industrial chemicals 1994;60:389- 433. Lyon: France. Available at: http://monographs.iarc.fr/ENG/Monographs/vol60/mono60.pdf [last accessed: 15/12/2014]

[5] Agency for Toxic Substances and Disease Registry [ATSDR]. *Toxicological profile of acrylamide.* U.S. Department of Health and Human Services. Public Health Service. December 2012. Available at: http://www.atsdr.cdc.gov/toxprofiles/tp203.pdf [last accessed: 09/01/2015].

[6] Friedman MA, Dulak LH, Stedham MA. *A lifetime oncogenicity study in rats with acrylamide.* Fund Appl Toxicol 1995;27:95-105.

[7] Joint Expert Committee on Food Additives [JECFA]. *Acrylamide.* In: *Evaluation of Certain contaminants in food, seventy-Second report of the Joint FAO/WHO Expert Committee on Food Additives*. WHO Technical Report Series 2011;959:9-21. Geneva, Switzerland: Available at: http://whqlibdoc.who.int/trs/who_trs_959_eng.pdf [last accessed: 03/02/2015].

[8] Johnson KA, Gorzinski SJ, Bodner KM, et al. *Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fisher 344 rats.* Toxicol Appl Pharm 1986;85:154-68.

[9] Raoufi A, Jamshidi K. *Acrylamide induced chronic nephrotoxicity in Rats.* Toxicol Lett 2013;221S:S59-S256.

[10] Baum M, Fauth E, Fritzen S, et al. *Acrylamide and glycidamide: genotoxic effects in V79-cells and human blood.* Mut Res 2005;580:61-9.

[11] Doerge DR, Gamboa da Costa G, McDaniel LP, et al. *DNA adducts derived from administration of acrylamide and glycidamide to mice and rats.* Mut Res 2005; 580:131-41.

[12] Gamboa da Costa G, Churchwell MI, Hamilton LP, et al. *DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice.* Chem Res Toxicol 2003;16:1328-37.

[13] Beland FA, Mellick PW, Olson GR, et al. *Carcinogenicity of acrylamide in B6C3F1 mice and F344/N rats from a 2-year drinking water exposure.* Food Chem Toxicol 2013;51:149-59.

[14] Blasiak J, Gloc E, Wozniak K, et al. *Genotoxicity of acrylamide in human lymphocytes.* Chem-Biol Interact 2004;149:137-49.

[15] Ghanayem BI, Witt KL, El-Hadri L, et al. *Comparison of germ cell mutagenicity in male CYP2E1-null and wildtype mice treated with acrylamide: evidence supporting a glycidamide mediated effect.* Biol Reprod 2005;72:157-63.

[16] Riboldi PB, Vinhas AM, Moreira JD. *Risks of dietary acrylamide exposure: A systematic review.* Food Chem 2014;157:310-22.

[17] World Health Organization [WHO]. *Summary report of the sixty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA*). Rome, 8-17 February 2005;7-17. Available at: ftp://ftp.fao.org/es/esn/jecfa/jecfa64_summary.pdf [last accessed: 10/12/2014].

[18] Mottram DS, Wedzicha BL, Dodson AT. *Acrylamide is formed in the Maillard reaction.* Nature 2002;419:448-9.

[19] Zyzak DV, Sanders RA, Stojanovic M, et al. *Acrylamide formation mechanism in heated foods.* J Agr Food Chem 2003;51:4782-7.

[20] Keramat J, Le Bail A, Prost C, et al. *Acrylamide in foods: Chemistry and analysis. A review.* Food Bioprocess Tech 2011;4:340-63.

[21] Joint Expert Committee on Food Additives [JECFA]. *Acrylamide.* In: *Evaluation of certain food contaminants, sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives.* WHO Technical Report 2006;930:8-26. Geneva, Switzerland. Available at: http://whqlibdoc.who.int/trs/WHO_TRS_930_eng.pdf [last accessed: 09/01/2015].

[22] Annola K, Keski-Rahkonen P, Vähäkangas K, et al. *Simultaneous determination of acrylamide, its metabolite glycidamide and antipyrine in human placental perfusion fluid and placental tissue by liquid chromatography-electrospray tandem mass spectrometry.* J Chromatograp B 2008;876:191-7.

[23] Capuano E, Fogliano V. *Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies.* LWT-Food Sci Technol 2011;44:793-810.

[24] Arribas-Lorenzo G, Morales FJ. *Recent insights in Acrylamide as carcinogen in foodstuffs.* Adv Mol Toxicol 2012;6:163-93.

[25] Bjellaas T, Stølen LH, Haugen M, et al. *Urinary acrylamide metabolites as biomarkers for short-term dietary exposure to acrylamide.* Food Chem Toxicol 2007;45:1020-6.

[26] Boettcher MI, Bolt HM, Drexler H, et al. *Excretion of mercapturic acids of acrylamide and glycidamide in human urine after single oral administration of deuterium-labelled acrylamide.* Arch Toxicol 2006;80:55-61.

[27] European Commission [EC]. *Recommendation of 3 May 2007 on the monitoring of acrylamide levels in food.* 2007/331/EC. OJ L 123/33, 12.5.2007. Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32007H0331&from=EN [last accessed: 10/03/2015].

[28] European Food Safety Authority [EFSA]. *Update on acrylamide levels in food from monitoring years 2007 to 2010.* EFSA Journal 2012;10:2938. doi:10.2903/j.efsa.2012.2938. Available at: http://www.efsa.europa.eu/it/efsajournal [last accessed: 20/01/2015].

[29] European Food Safety Authority. EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2015. *Scientific Opinion on acrylamide in food.* EFSA Journal 2015;13:4104, 321. doi:10.2903/j.efsa.2015.4104. Available at: www.efsa.europa.eu/efsajournal [last accessed: 05/06/2015].

[30 Freisling H, Moskal A, Ferrari P, et al. *Dietary acrylamide intake of adults in the European Prospective Investigation into Cancer and Nutrition differs greatly according to geographical region.* Eur J Nutr 2013;52:1369-80.

[31] Carrieri G, Anese M, Quarta B, et al. *Evaluation of acrylamide formation in potatoes during deep-frying: The effect of operation and configuration.* J Food Eng 2010;98:141-9.

[32] Marchettini N, Focardi S, Guarnieri M, et al. *Determination of acrylamide in local and commercial cultivar of potatoes from biological farm.* Food Chem 2013;136:1426-8.

[33] Tateo F, Bononi M, Gallone F. *Acrylamide content in potato chips on the Italian market determined by liquid chromatography tandem mass spectrometry.* Int J Food Sci Tech 2010;45:629-34.

[34] Banchero M, Pellegrino G, Manna L. *Supercritical fluid extraction as a potential mitigation strategy for the reduction of acrylamide level in coffee.* J Food Eng 2013;115:292-7.

[35] World Health Organization [WHO]. *Guidelines for Drinking-Water Quality, incorporating 1st and 2nd addenda,* Vol. 1, Recommendations. 3rd ed. 2008; Geneva, Switzerland. Available at: http://www.who.int/water_sanitation_health/dwq/fulltext.pdf?ua=1 [last accessed: 12/01/2015].

[36] European Commission [EC]. *Recommendation of 10 January 2011 on investigations into the levels of acrylamide in food,* C (2010) 9681 final 2011. Available at: http://ec.europa.eu/food/food/chemicalsafety/contaminants/recommendation_10012011_acrylamide_food_en.pdf [last accessed: 19/01/2015].

[37] European Commission [EC]. *Recommendation of 8 November 2013 on investigations into the levels of acrylamide in food.* 2013/647/EU. OJ L 301/15, 12.11.2013. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:301:FULL:EN:PDF [last accessed: 05/12/2014].

[38] European Commission [EC]. *Commission recommendations of 2 June 2010 on the monitoring of acrylamide levels in food.* 2010/307/EU. Official Journal of the European Union, L137, 3.6.2010; 4-10. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:137:0004:0010:EN:PDF [last accessed: 0512/2014].

[39] Istituto di Servizi per il Mercato Agricolo Alimentare [ISMEA]. *Food consumptions – Italian household consumptions* [in Italian]. No. 1/13. 2013. Available at: http://www.ismea.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/8499 [last accessed: 12/01/2015].

[40] Varacca A, Sckokai P. *Product differentiation and competition between brands in market for breakfast cereals* [in Italian]. Agriregionieuropa 2012;8:31. Available at: http://hdl.handle.net/10807/39180 [last accessed: 12/01/2015].

[41] Wang H, Feng F, Guo Y, et al. *HPLC-UV quantitative analysis of acrylamide in baked and deep-fried Chinese foods.* J Food Compos Anal 2013;31:7-11.

[42] Gökmen V, Morales FJ, Ataç B, et al. *Multiple-stage extraction strategy for the determination of acrylamide in foods.* J Food Compos Anal 2009;22:142-7.

[43] Anese M, Quarta B, Peloux L, et al. *Effect of formulation on the capacity of l-asparaginase to minimize acrylamide formation in short dough biscuits.* Food Res Int 2011;44:2837-42.

[44] Russo MV, Avino P, Centola A, et al. *Rapid and simple determination of acrylamide in conventional cereal-based foods and potato chips through conversion to 3-[bis(trifluoroethanoyl) amino]-3-oxopropyl trifluoroacetate by gas chromatography-coupled with electron capture and ion trap mass spectrometry detectors.* Food Chem 2014;146:204-11.

[45] Yusa V, Quintas G, Pardo O, et al. *Determination of acrylamide in foods by pressurized fluid extraction and liquid chromatography-tandem mass spectrometry used for a survey of Spanish cereal-based foods.* Food Addit Contam 2006;23:237-44.

[46] Ren Y, Zhang Y, Jiao J, et al. *Sensitive isotope dilution liquid chromatography/electrospray ionization tandem mass spectrometry method for the determination of acrylamide in chocolate.* Food Addit Contam 2006;23:228-36.

[47] Sirot V, Hommetb F, Tard A, et al. *Dietary acrylamide exposure of the French population: Results of the second French Total Diet Study.* Food Chem Toxicol 2012; 50:889-94.

[48] Leclercq C, Arcella D, Piccinelli R, et al. on behalf of the INRAN-SCAI 2005-06 Study Group. *The Italian food consumption survey INRAN-SCAI 2005–06: main results in terms of food consumption*. Public Health Nutr 2009;12:2504-32. Available at: http://nut.entecra.it/710/I_consumi_alimentari__INRAN-SCAI_2005-06.html. [last accessed: 22/06/2015].

■ Received on June 9, 2015. Accepted on November 9, 2015.

■ Correspondence: Raffaella Capei, Department of Health Sciences, University of Florence, viale Morgagni 48, 50134 Florence, Italy - Tel. +39 055 2751078 - E-mail: raffaella.capei@unifi.it